CLAIMS

WHAT IS CLAIMED IS:

1. A process for inducing direct somatic embryogenesis in monocotyledonous plant cells and rapidly regenerating fertile monocotyledonous plants, comprising the steps of:

(a) culturing embryogenic monocotyledonous plant cells under conditions conducive to direct formation of primary embryos without an intervening callus stage, at least until at least one primary embryo reaches the globular developmental stage; and one of the following steps selected from:

(b) culturing one or more of the primary embryos from step (a) under conditions conducive to regeneration of plantlets from the primary embryos, and culturing the primary embryo in or on a regeneration medium;

(c) culturing one or more of the primary embryos from step (a) under conditions conducive to induction of secondary embryo formation, at least until secondary embryogenesis is detected, and culturing one or more of the secondary embryos under conditions conducive to regeneration of plantlets from the secondary embryos; or

(d) culturing one or more of the primary embryos from step (a) under conditions conducive to induction of organogenesis, at least until adventitious shoots are detected; and culturing the adventitious shoots under conditions conducive to regeneration of plantlets.

2. The process of claim 1, wherein step (a) further comprises culturing the embryogenic cells in or on a culture medium comprising auxin, cytokinin and polyamine in amounts effective to cause direct formation of primary embryos without an intervening callus stage, the auxin being present in greater proportion than the cytokinin.

3. The process of claim 2, wherein, in step (a), the ratio of auxin to cytokinin in the culture medium is from about 5 μM auxin per 1 μM cytokinin to about 20 μM auxin per 1 μM cytokinin.

4. The process of claim 3, wherein, in step (a) the culture medium includes the plant growth regulators:

i) from about 15 μM auxin to about 45 μM auxin;

ii) from about 15 μM polyamine to about 45 μM polyamine; and

iii) from about 1 μM cytokinin to about 5 μM cytokinin.

5. The process of claim 2, wherein, in step (a), the ratio of auxin to cytokinin in the culture medium is about 14 μM auxin per 1 μM cytokinin.

6. The process of claim 5, wherein, in step (a), the culture medium includes the plant growth regulators of:

i) about 30 μM auxin;

ii) about 30 μM polyamine; and

iii) about 2 μM cytokinin.

7. The process of claim 2, wherein, in step (a), the culture medium is DSEM medium.

8. The process of claim 4, wherein steps (a) and (b) are conducted, and further comprise culturing the primary embryo in or on a regeneration medium.

9. The process of claim 4, wherein steps (a) and (c) are conducted, and further comprise culturing the primary embryo in or on a culture medium comprising auxin, cytokinin, and polyamine in amounts effective to cause induction of secondary embryo formation, the cytokinin being present in greater proportion than the auxin.

10. The process of claim 4, wherein steps (a) and (d) are conducted, and further comprise culturing the primary embryo in or on a culture medium comprising auxin, cytokinin, and polyamine in amounts effective to cause induction of organogenesis, the cytokinin being present in greater proportion than the auxin.

11. The process of claim 8, wherein the regeneration medium is MS medium.

12. The process of claim 8, further comprising, before step (b), the step of culturing the primary embryo under conditions conducive to germination of the primary embryos until germination of at least one of the primary embryos commences.

13. The process of claim 12, wherein the germination step comprises culturing the primary embryo in or on a culture medium which comprises polyamine in an amount effective to cause germination of the primary embryos, and which is essentially free of either auxin or cytokinin.

14. The process of claim 13, wherein the culture medium comprises from about 15 μM polyamine to about 45 μM polyamine.

15. The process of claim 13, wherein the culture medium comprises about 30 μM polyamine.

16. The process of claim 13, wherein the germination step comprises culturing the primary embryo in or on GEM medium.

17. The process of claim 8, further comprising the step of culturing the plantlets under conditions conducive to induction of root formation until the plantlets form roots.

18. The process of claim 17, further comprising the step of transplanting the plantlets to soil and growing them to maturity.

19. The process of claim 18, wherein the embryogenic cells are Poaceae embryogenic cells, and wherein the cells are selected from the genera consisting of *Triticum*, *Hordeum*, *Secale*, *Avena*, *Zea*, *Oryza*, *Sorghum*, *Pennesitum*, *Saccharum*, *Dactylis*, *Bromus*, and *Lolium*.

20. The process of claim 18, wherein the embryogenic cells are Liliaceae embryogenic cells, and wherein the cells are selected from the genus *Allium*.

21. The process of claim 18, wherein the embryogenic cells are selected from the group consisting of *Hordeum vulgare*, *Triticum aestivum, Triticum durum, Triticum monococum, Triticum urartu, Secale cereale, Avena sativa* and *Triticum durum amphiploids* embryogenic cells.

22. The process of claim 19, 20, or 21, wherein the embryogenic cells of step (a) are scutella cells.

23. The process of claim 19, 20, or 21, wherein the embryogenic cells of step (a) are scutella cells free of a germ.

24. The process of claim 23, which further includes, after step (a), cutting the scutellum carrying the primary embryo into a plurality of pieces prior to culturing in step (b).

25. The process of claim 24, wherein the scutellum carrying the primary embryo is cut into two to four pieces.

26. The process of claim 19, 20, or 21, wherein step (a) further comprises the step of introducing foreign DNA into the embryogenic cells or primary embryo so that the foreign DNA becomes stably integrated into the genome of the cells.

27. The process of claim 26, wherein the foreign DNA is introduced into the embryogenic cells or primary embryo by particle bombardment or by *Agrobacterium*-mediated transformation.

28. The process of claim 27, wherein the foreign DNA is introduced into the embryogenic cells or primary embryo in step (a) during the development of the primary embryo.

29. The process of claim 28, wherein the foreign DNA is introduced into the embryogenic cells between zero to five days after commencement of tissue culture.

30. The process of claim 28, wherein the foreign DNA is introduced into the embryogenic cells or the primary embryo after two days following commencement of tissue culture.

31. The process of claim 28, wherein after the foreign DNA has been introduced, the embryogenic cells or primary embryo are transferred to a media for steps (a) and (b) which includes a selective agent to identify a transformed plant cell that has incorporated the foreign DNA.

32. The process of claim 31, wherein transformed plant cells are cultured in media to support regeneration of transformants.

33. The process of claim 32, which further comprises confirming expression of the foreign DNA in the transformed plants by one or both of polymerase chain reaction and Southern blot analyses.

34. The process of claim 9, wherein, in step (c), the ratio of auxin to cytokinin in the culture medium is from about 0.05 μM auxin per 1 μM cytokinin to about 0.2 μM auxin per 1 μM cytokinin.

35. The process of claim 34, wherein, in step (c), the culture medium includes the plant growth regulators:

i) from about 5 μM auxin to about 15 μM auxin;

ii) from about 15 μM polyamine to about 45 μM polyamine; and

iii) from about 50 μM cytokinin to about 200 μM cytokinin.

36. The process of claim 9, wherein, in step (c) the ratio of auxin to cytokinin is about 0.1 μM auxin per 1.0 μM cytokinin.

37. The process of claim 36, wherein, in step (c), the culture medium includes the plant growth regulators of:

i) about 11 μM auxin;

ii) about 30 μM polyamine; and

iii) about 110 μM cytokinin.

38. The process of claim 9, wherein, in step (c), the culture medium is SEM medium.

39. The process of claim 38, wherein step (c) comprises culturing the secondary embryo in or on a regeneration medium.

40. The process of claim 39, wherein the regeneration medium is MS medium.

41. The process of claim 39, further comprising, before step (c), the step of culturing the secondary embryo under conditions conducive to germination of the secondary embryos until germination of at least one of the secondary embryos commences.

42. The process of claim 41, wherein the germination step comprises culturing the secondary embryo in or on a culture medium which comprises polyamine in an amount effective to cause germination of the secondary embryos, and which is essentially free of either auxin or cytokinin.

43. The process of claim 42, wherein the culture medium comprises from about 15 μM polyamine to about 45 μM polyamine.

44. The process of claim 42, wherein the culture medium comprises about 30 μM polyamine.

45. The process of claim 42, wherein the germination step comprises culturing the secondary embryo in or on GEM medium.

46. The process of claim 39, further comprising the step of culturing the plantlets under conditions conducive to induction of root formation until the plantlets form roots.

47. The process of claim 46, further comprising the step of transplanting the plantlets to soil and growing them to maturity.

48. The process of claim 47, wherein the embryogenic cells are Poaceae embryogenic cells, and wherein the cells are selected from the genera consisting of *Triticum*, *Hordeum*, *Secale*, *Avena*, *Zea*, *Oryza*, *Sorghum*, *Pennesitum*, *Saccharum*, *Dactylis*, *Bromus*, and *Lolium*.

49. The process of claim 47, wherein the embryogenic cells are Liliaceae embryogenic cells, and wherein the cells are selected from the genus *Allium*.

50. The process of claim 47, wherein the embryogenic cells are selected from the group consisting of *Hordeum vulgare*, *Triticum aestivum, Triticum durum, Triticum monococum, Triticum urartu, Secale cereale, Avena sativa* and *Triticum durum amphiploids* embryogenic cells.

51. The process of claim 48, 49, or 50, wherein the embryogenic cells of step (a) are scutella cells.

52. The process of claim 48, 49, or 50, wherein the embryogenic cells of step (a) are scutella cells free of a germ.

53. The process of claim 52, which further includes, after step (a), cutting the scutellum carrying the primary embryo into a plurality of pieces before culturing in step (c).

54. The process of claim 53, wherein the scutellum carrying the primary embryo is cut into two to four pieces.

55. The process of claim 53, which further comprises, before step (c), the step of cutting the primary embryo carrying the secondary embryo into a plurality of pieces to obtain a high frequency of germination of secondary embryo.

56. The process of claim 55, wherein the primary embryos carrying the secondary embryo is cut into two pieces.

57. The process of claim 48, 49, or 50, wherein step (a) further comprises the step of introducing foreign DNA into the embryogenic cells or the primary embryo so that the foreign DNA becomes stably integrated into the genome of the cells.

58. The process of claim 57, wherein the foreign DNA is introduced into the embryogenic cells or primary embryo by particle bombardment or by *Agrobacterium*-mediated transformation.

59. The process of claim 58, wherein the foreign DNA is introduced into the embryogenic cells or the primary embryo in step (a) during the development of the primary embryo.

60. The process of claim 59, wherein the foreign DNA is introduced into the embryogenic cells or the primary embryo between zero to five days after commencement of tissue culture.

61. The process of claim 59, wherein the foreign DNA is introduced into the embryogenic cells or the primary embryo after two days following commencement of tissue culture.

62. The process of claim 59, wherein after the foreign DNA has been introduced, the embryogenic cells or primary embryo are transferred to a media for step (c), and optionally for step (a), which includes a selective agent to identify a transformed plant cell that has incorporated the foreign DNA.

63. The process of claim 62, wherein transformed plant cells are cultured in media to support regeneration of transformants.

64. The process of claim 63, which further comprises confirming expression of the foreign DNA in the transformed plants by one or both of polymerase chain reaction and Southern blot analyses.

65. The process of claim 10, wherein, in step (d), the ratio of auxin to cytokinin in the culture medium is from about 0.05 μM auxin per 1 μM cytokinin to about 0.2 μM auxin per 1 μM cytokinin.

66. The process of claim 65, wherein, in step (d), the culture medium includes the plant growth regulators:

i) from about 5 μM auxin to about 15 μM auxin;

ii) from about 15 μM polyamine to about 45 μM polyamine; and

iii) from about 50 μM cytokinin to about 200 μM cytokinin.

67. The process of claim 10, wherein, in step (d) the ratio of auxin to cytokinin is about 0.1 μM auxin per 1.0 μM cytokinin.

68. The process of claim 67, wherein, in step (d), the culture medium includes the plant growth regulators of:

i) about 11 μM auxin;

ii) about 30 μM polyamine; and

iii) about 110 μM cytokinin.

69. The process of claim 10, wherein, in step (d), the culture medium is SEM medium.

70. The process of claim 66, wherein step (d) comprises culturing the new shoots in or on a regeneration medium.

71. The process of claim 70, wherein the regeneration medium is MS medium.

72. The process of claim 70, further comprising the step of culturing the plantlets and shoots under conditions conducive to induction of root formation until the plantlets form roots.

73. The process of claim 72, comprising the further step of transplanting the plantlets to soil and growing them to maturity.

74. The process of claim 73, wherein the embryogenic cells are Poaceae embryogenic cells, and wherein the cells are selected from the genera consisting of *Triticum*, *Hordeum*, *Secale*, *Avena*, *Zea*, *Oryza*, *Sorghum*, *Pennesitum*, *Saccharum*, *Dactylis*, *Bromus*, and *Lolium*.

75. The process of claim 73, wherein the embryogenic cells are Liliaceae embryogenic cells, and wherein the cells are selected from the genus *Allium*.

76. The process of claim 73, wherein the embryogenic cells are selected from the group consisting of *Zea mays* and *Sorghum bicolor*.

77. The process of claim 74, 75, or 76, wherein the embryogenic cells of step (a) are scutella cells.

78. The process of claim 74, 75, or 76, wherein the embryogenic cells of step (a) are scutella cells free of a germ.

79. The process of claim 78, which further includes, after step (a), cutting the scutellum carrying the primary embryo into a plurality of pieces before culturing in step (d).

80. The process of claim 79, wherein the scutellum carrying the primary embryo is cut into two to four pieces.

81. The process of claim 74, 75, or 76, wherein step (a) further comprises introducing foreign DNA into the embryogenic cells or the primary embryo so that the foreign DNA becomes stably integrated into the genome of the cells.

82. The process of claim 81, wherein the foreign DNA is introduced into the embryogenic cells or primary embryo by particle bombardment or by *Agrobacterium*-mediated transformation.

83. The process of claim 82, wherein the foreign DNA is introduced into the embryogenic cells or primary embryo in step (a) during the development of the primary embryo.

84. The process of claim 83, wherein the foreign DNA is introduced into the embryogenic cells between zero to five days after commencement of tissue culture.

85. The process of claim 83, wherein the foreign DNA is introduced into the embryogenic cells or the primary embryo after two days following commencement of tissue culture.

86. The process of claim 83, wherein after the foreign DNA has been introduced, the embryogenic cells or primary embryo are transferred to a media for steps (a) and (d) which includes a selective agent to identify a transformed plant cell that has incorporated the foreign DNA.

87. The process of claim 86, wherein transformed plant cells are cultured in media to support regeneration of transformants.

88. The process of claim 87, which further comprises confirming expression of the foreign DNA in the transformed plants by one or both of polymerase chain reaction and Southern blot analyses.

89. A process for inducing somatic embryogenesis in monocotyledonous callus cells, suspension cells, or microspore-derived embryos, and rapidly regenerating fertile monocotyledonous plants, comprising the steps of:

(a) culturing embryogenic monocotyledonous callus cells, suspension cells or microspore-derived embryos in or on a culture medium comprising auxin, cytokinin, and polyamine in amounts effective to cause induction of embryo formation, the cytokinin being present in greater proportion than the auxin, at least until at least one embryo reaches the globular developmental stage; and

(b) cultivating the one or more globular-stage embryos from step (a) under conditions conducive to regeneration of plantlets.

90. The process of claim 89, wherein, in step (a), the ratio of auxin to cytokinin in the culture medium is from about 0.05 μM auxin per 1 μM cytokinin to about 0.2 μM auxin per 1 μM cytokinin.

91. The process of claim 90, wherein, in step (a), the culture medium includes the plant growth regulators:

i) from about 5 μM auxin to about 15 μM auxin;

ii) from about 15 μM polyamine to about 45 μM polyamine; and

iii) from about 50 μM cytokinin to about 200 μM cytokinin.

92. The process of claim 89, wherein, in step (a) the ratio of auxin to cytokinin is about 0.1 μM auxin per 1.0 μM cytokinin.

93. The process of claim 92, wherein, in step (a), the culture medium includes the plant growth regulators:

i) about 11 μM auxin;

ii) about 30 μM polyamine; and

iii) about 110 μM cytokinin.

94. The process of claim 89, wherein, in step (a), the culture medium is SEM medium.

95. The process of claim 91, wherein step (b) comprises culturing the embryo in or on a regeneration medium.

96. The process of claim 95, wherein the regeneration medium is MS medium.

97. The process of claim 95, further comprising the step of (c) culturing the plantlets under conditions conducive to induction of root formation until the plantlets form roots.

98. The process of claim 97, further comprising the step of (d) transplanting the plantlets to soil and growing them to maturity.

99. The process of claim 98, wherein the embryogenic monocotyledonous callus cells, suspension cells or microspore-derived embryos are of Poaceae and are selected from the genera consisting of *Triticum*, *Hordeum*, *Secale*, *Avena*, *Zea*, *Oryza*, *Sorghum*, *Pennesitum*, *Saccharum*, *Dactylis*, *Bromus*, and *Lolium*.

100. The process of claim 99, wherein the embryogenic monocotyledonous callus cells, suspension cells or microspore-derived embryos are of Liliaceae and from the genus *Allium*.

101. The process of claim 100, wherein the embryogenic monocotyledonous callus cells, suspension cells or microspore-derived embryos are selected from the group consisting of *Hordeum vulgare*, *Triticum aestivum, Triticum durum, Triticum monococum, Triticum urartu, Secale cereale, Avena sativa* and *Triticum durum amphiploids*.

102. The process of claim 99, 100, or 101, which further comprises, before step (a), introducing foreign DNA into the embryogenic monocotyledonous callus cells, suspension cells or microspore-derived embryos so that the foreign DNA becomes stably integrated into the genome of the cells or embryos.

103. The process of claim 102, wherein the foreign DNA is introduced into the embryogenic monocotyledonous callus cells, suspension cells or microspore-derived embryos by particle bombardment or by *Agrobacterium*-mediated transformation.

104. The process of claim 103, wherein after the foreign DNA has been introduced, the embryogenic monocotyledonous callus cells, suspension cells or microspore-derived embryos are transferred to a media for steps (a) and (b) which includes a selective agent to identify a transformed plant cell that has incorporated the foreign DNA.

105. The process of claim 104, wherein transformed plant cells are cultured in media to support regeneration of transformants.

106. The process of claim 105, which further comprises confirming expression of the foreign DNA in the transformed plants by one or both of polymerase chain reaction and Southern blot analyses.